[redacted]17-7465/002:

Artificially aged, artificially decomposed, artificially degraded mixed micelles. Made by exposure of Ro/001 to a temperature of 80°C for 250-264 hours resulting in hydrolysis of ~ 25% lecithin into lysolecithin (~ 40 mg/ml) and fatty acids (~ 20 mg/ml).

[redacted]17-7465/003:

Stored mixed micelles. [redacted]17-7465/001 stored for 4 months at 35 °C resulting in hydrolysis of ~ 5% lecithin into lysolecithin (~ 8 mg/ml) and fatty acids (~ 4 mg/ml).

ACUTE TOXICITY

Acute Studies performed:

1. Single dose study in mice with fresh mixed micelles injected IV at a slow and fast rate. (ref IIIA1)
2. Acute IV toxicity study of mixed micelle samples with different peroxide numbers of lecithin in male rats. (ref. III A2)
3. 5-dose IV study (5 doses administered once/day for 5 days) in mice and rats with fresh mixed micelles. (ref. III A3)
4. 5-dose IV study (5 doses administered once/day for 5 days) in mice and rats with stored mixed micelles. (ref. III A4).
5. A single dose study in rabbits with fresh and stored mixed micelles. (ref. III A5)

SPECIES	SEX	INJECTION RATE	CONDITION OF MIXED MICELLES	LD50 (ml/kg) AFTER 1ST DOSE (HRS AFTER LAST DOSE)	LD50 (ml/kg) AFTER 5TH DOSE (HRS AFTER LAST DOSE)
MOUSE	M	SLOW	FRESH	5.9 (72h)	
	M	FAST	FRESH	5.9 (72 h)	
	M/F		FRESH	5.5 (24 h)	5.5 (240 h)
	M/F		STORED 6 MO 4°C	5.5 (24 h)	5.1 (240 h)
	M/F		STORED 6 MO 22°C	4.9 (24 h)	3.5 (240 h)
	M/F		STORED 6 MO 35°C	5.2 (24 h)	3.5 (240 h)
			STORED 6 MO 45°C	2.7 (24 h)	2.6 (240 h)
RAT	M/F		FRESH	7.1 (24 h)	7.1 (240 h)
	M		FRESH PEROXIDE NO. 1.7	4.8 (24 h)	
	M		FRESH PEROXIDE NO 13	4.7 (24 h)	
	M		FRESH PEROXIDE NO 18.5	4.9 (24 h)	
	M/F		STORED 6 MO 4°C	7.1 (24 h)	7.1 (240 h)
	M/F		STORED 6 MO 22°C	7.1 (24 h)	7.1 (240 h)
	M/F		STORED 6 MO 35°C	4.6 (24 h)	3.5 (240 h)
	M/F		STORED 6 MO 45°C	3.5 (24 h)	3.5 (240 h)
RABBIT	M		FRESH	5.9 (24 h)	
	M		FRESH	5.3 (24 h)	
	M		STORED 6 MO 4°C	4.2 (24 h)	
	M		STORED 6 MO 4°C	4.2 (24 h)	
	M		STORED 6 MO 22°C	3.5 (24 h)	
	M		STORED 6 MO 22°C	3.5 (24 h)	
	M		STORED 6 MO 35°C	3.4 (24 h)	
	M		STORED 6 MO 35°C	3.4 (24 h)	
	M		STORED 6 MO 45°C	2.0 (24 h)	
	M		STORED 6 MO 45°C	2.0 (24 h)	

SUMMARY OF ACUTE STUDIES

Toxic symptoms in mice were respiratory depression and relaxation at low doses; higher doses produced ataxia at a slow injection rate of lethal doses; death was preceded by clonic convulsions whereas no convulsions were observed at a fast injection rate. Similar symptoms

were observed in rats. Death was preceded by convulsions at highly toxic doses and ataxia, respiratory depression and relaxation with lower doses. In one of the rat studies, blood samples taken from 24 h survivors showed markedly elevated plasma transaminases (ALAT, ASAT) whereas cellular hematological parameters were not modified. In rabbits the clinical signs were less marked, only slight sedation was noted at a 75% lethal dose. The calculated LD50 values of fresh or mixed micelles stored for 6 months at 4° or 22°C ranged in mice and rabbits between 3.5 and 5.9 ml/kg. In rats, these values were between 4.7 and 7.1 mg/kg. Expressed in relation of the toxic constituent (glycocholic acid) 3.5 ml/kg corresponds to 310 mg/kg of glycocholic acid, 5.9 ml/kg corresponds to 522 mg/kg and 7.1 ml/kg corresponds to 628 mg/kg, respectively. An IV administration of glycocholic acid alone produced an LD50 of ~ 320 mg/kg which corresponds well with the calculated value from the mixed micelle studies. Mixed micelles were about equally toxic in the three species studied.

MULTIPLE DOSE TOXICOLOGY STUDIES

Based on the results of the 5 day administration studies, there did not appear to be a cumulative toxic effect and the injection rate did not affect the lethality values. It is proposed by the sponsor that the administration period is not likely to exceed 7 days. Therefore, the toxicology program included up to 4 weeks of administration in rats and dogs.

Toxicity was significantly increased after storage of the mixed micellar solution at higher ambient temperatures. The sponsor attributes this to the partial degradation of lecithin to lysolecithin and fatty acid.

FOUR WEEK INTRAVENOUS TOXICITY STUDY IN RATS WITH FRESH AND ARTIFICIALLY DECOMPOSED MIXED MICELLES (REFS III B1, IIIC5)

[redacted]

PURPOSE: To determine the toxicity of fresh and artificially decomposed mixed micelles in rats after 4 weeks of daily administration IV.

EXPERIMENTAL DESIGN: SPF-bred, Wistar-derived Fü-albino rats were randomly assigned 12/sex/group which received daily doses of:
2.25 mg/kg physiological saline (controls)
0.25, 0.75 and 2.25 ml/kg of undecomposed mixed micellar solvent
0.5 and 2.25 mg/kg decomposed mixed micelles.

Following 4 weeks of treatment, 6 rats/sex of the controls and both high dose groups were necropsied; the remaining animals were necropsied after a 2 week drug free observation period. All animals of the low and mid dose groups were necropsied after the initial 4 weeks. High dose corresponds to approximately a 100 ml bolus injection in humans.

RESULTS

MORTALITY: 8 rats died prematurely. 6 females: 2 controls, 2 in HD [redacted] 17-7465/001, 1 in each [redacted] 17-7465/002 group. These deaths were not considered drug-related. Two male rats died at the beginning of week 3 (1 in HD [redacted] 17-7465/001 and one in HD [redacted] 17-7465/002).

OBSERVED EFFECTS: Occasional dizziness and drowsiness immediately after injection of the undecomposed mixed micelles at the LD and MD early in the study. This subsided as the study progressed. Similar findings were observed with the high dose of decomposed compound.

BODY WEIGHT: Sponsor indicated no clear treatment-related effect. However, in males, there was approximately a 5% decrease in HD (001) group and a 7% decrease in HD (002) group compared to controls at day 30. These changes recovered during the postdosing period. There were no biologically significant effects in females or lower dose males.

FOOD CONSUMPTION: No data.

VITAL SIGNS: No data.

OPHTHALMIC EXAMINATION: No findings. (no data presented).

HEMATOLOGY: Slight increase in reticulocyte counts in HD 001 males and females at 4 weeks. A more significant finding occurred in HD males and females (002) which recovered after dosing stopped; Howell-Jolly body formation (micronuclei) was detected in the HD decomposed micelle group for both males and females (all animals had elevations). These findings were reversible except in one male and one female in the HD /002 group. It is not clear if this was performed to the specifications that might indicate this was a clastogenic finding. This was not reported in any other toxicology study in any other species. The significance of the finding of Howell-Jolly bodies in this study is unclear.

COAGULATION: No data.

BONE MARROW: No data.

BLOOD CHEMISTRY: In HD animals of either material, GOT and GPT were slightly but consistently elevated throughout the study. ALK PHOS was generally, but not always slightly elevated. It is not clear if the ALK PHOS findings are significant treatment-related findings. Findings were evident by the end of the first week of the study. These elevations were completely reversible after 2 weeks of non-treatment. Mild to moderate increases in plasma bilirubin were noted in HD animals treated with [redacted] 17-7465/001 while these findings were moderate to marked for [redacted] 17-7465/002.